

What Is Claimed Is:

1. A serum-free cell culture medium comprising a fibroblast growth factor (FGF) and an agent causing an increase in intracellular levels of cyclic adenosine monophosphate (cAMP), wherein said medium is capable of supporting the cultivation of an animal epithelial cell *in vitro*.

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2. The medium of claim 1, wherein said FGF is selected from the group consisting of FGF-1 (aFGF), FGF-2 (bFGF) and FGF-7 (KGF).

3. The medium of claim 2, wherein said FGF is aFGF.

4. The medium of claim 1, wherein said agent causing an increase in intracellular levels of cAMP functions through interaction with a cellular G-protein.

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5. The medium of claim 1, wherein said agent causing an increase in intracellular levels of cAMP functions by directly increasing intracellular cAMP levels.

6. The medium of claim 1, wherein said agent causing an increase in intracellular levels of cAMP functions by inhibiting a cAMP phosphodiesterase.

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7. The medium of claim 1, wherein said agent causing an increase in intracellular levels of cAMP is a β -adrenergic receptor agonist.

8. The medium of claim 4, wherein said agent is cholera toxin or forskolin.

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9. The medium of claim 5, wherein said agent is dibutyryl cAMP.

10. The medium of claim 6, wherein said agent is isobutylmethylxanthine or theophylline.

11. The medium of claim 7, wherein said agent is isoproterenol.

5 12. The medium of claim 1, said medium further comprising ascorbic acid.

13. The medium of claim 1, wherein said medium is a 1X medium formulation.

10 14. The cell culture medium of claim 1, wherein said medium formulation is a 10X concentrated medium formulation.

15 15. The cell culture medium of claim 1, said medium further comprising one or more ingredients selected from the group of ingredients consisting of an amino acid, a vitamin, an inorganic salt, adenine, ethanolamine, D-glucose, epidermal growth factor (EGF), heparin, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), hydrocortisone, insulin, lipoic acid, phenol red, phosphoethanolamine, putrescine, sodium pyruvate, T3, thymidine and transferrin.

16. The medium of claim 15, said medium further comprising ascorbic acid.

20 17. The cell culture medium of claim 15, wherein said amino acid ingredient comprises one or more amino acids selected from the group consisting

of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

5 18. The cell culture medium of claim 15, wherein said vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca⁺⁺-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B₁₂.

10 19. The cell culture medium of claim 15, wherein said inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of a calcium salt, CuSO₄, FeSO₄, KCl, a magnesium salt, a manganese salt, sodium acetate, NaCl, NaHCO₃, Na₂HPO₄, Na₂SO₄, a selenium salt, a silicon salt, a molybdenum salt, a vanadium salt, a nickel salt, a tin salt and a zinc salt.

15 20. A cell culture medium comprising the ingredients adenine, ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), hydrocortisone, insulin, lipoic acid, phenol red, phosphoethanolamine, putrescine, sodium pyruvate, T3, thymidine, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-
20 proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca⁺⁺-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B₁₂, a calcium salt, CuSO₄, FeSO₄, KCl, a magnesium salt, a manganese salt, sodium acetate, NaCl, NaHCO₃, Na₂HPO₄, Na₂SO₄, a selenium salt, a silicon salt, a molybdenum salt, a vanadium salt, a nickel salt, a tin
25 salt, and a zinc salt,

wherein each ingredient is present in an amount which supports the cultivation of an animal epithelial cell *in vitro*.

21. The medium of claim 20, said medium further comprising heparin, epidermal growth factor (EGF), a fibroblast growth factor (FGF) and an agent causing an increase in intracellular levels of cyclic adenosine monophosphate (cAMP).

22. The medium of claim 21, said medium further comprising ascorbic acid.

23. A cell culture medium obtained by combining a basal medium with heparin, EGF, a fibroblast growth factor (FGF) and an agent causing an increase in intracellular levels of cyclic adenosine monophosphate (cAMP), wherein said medium is capable of supporting the cultivation of an animal epithelial cell *in vitro*.

24. The medium obtained according to claim 23, wherein said FGF is selected from the group consisting of FGF-1 (aFGF), FGF-2 (bFGF) and FGF-7 (KGF).

25. The medium obtained according to claim 24, wherein said FGF is aFGF.

26. The medium obtained according to claim 23, wherein said agent causing an increase in intracellular levels of cAMP is an agent which functions through G-protein interaction.

27. The medium obtained according to claim 23, wherein said agent causing an increase in intracellular levels of cAMP is an agent which functions by directly increasing intracellular cAMP levels.

5 28. The medium obtained according to claim 23, wherein said agent causing an increase in intracellular levels of cAMP is an agent which functions by inhibiting a cAMP phosphodiesterase.

29. The medium obtained according to claim 23, wherein said agent causing an increase in intracellular levels of cAMP is an agent which is a β -adrenergic receptor agonist.

10 30. The medium of claim 26, wherein said agent is cholera toxin or forskolin.

31. The medium of claim 27, wherein said agent is dibutyryl cAMP.

32. The medium of claim 28, wherein said agent is isobutylmethylxanthine or theophylline.

15 33. The medium of claim 29, wherein said agent is isoproterenol.

20 34. The animal cell culture medium obtained according to claim 23, wherein said basal medium is obtained by combining one or more additional ingredients selected from the group consisting of adenine, ethanolamine, D-glucose, epidermal growth factor (EGF), heparin, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), hydrocortisone, insulin, lipoic acid, phenol red, phosphoethanolamine, putrescine, sodium pyruvate, T3, thymidine, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-

cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca⁺⁺-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B₁₂, a calcium salt, CuSO₄, FeSO₄, KCl, a magnesium salt, a manganese salt, sodium acetate, NaCl, NaHCO₃, Na₂HPO₄, Na₂SO₄, a selenium salt, a silicon salt, a molybdenum salt, a vanadium salt, a nickel salt, a tin salt and a zinc salt,

wherein each ingredient is added in an amount which supports the cultivation of an animal epithelial cell *in vitro*.

35. A cell culture medium obtained by combining the medium obtained according to either claim 23 or claim 34 and ascorbic acid.

36. The medium of any one of claims 1, 21, 23 or 34, wherein said animal epithelial cell is selected from the group of animal epithelial cells consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell and a tracheal epithelial cell.

37. The cell culture medium of claim 36, wherein said cell is a human cell.

38. The cell culture medium of claim 36, wherein said cell is a normal cell.

39. The cell culture medium of claim 36, wherein said cell is an abnormal cell.

40. The cell culture medium of claim 39, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

5 41. A method of cultivating an animal epithelial cell comprising the steps of

(a) contacting said cell with the cell culture medium of any one of claims 1, 21, 22, 23 or 34; and

(b) cultivating said cell under conditions suitable to support cultivation of said cell.

10 42. The method of claim 41, wherein said animal epithelial cell is selected from the group of animal epithelial cells consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell and a tracheal epithelial cell.

43. The method of claim 41, wherein said cell is a human cell.

44. The method of claim 41, wherein said cell is a normal cell.

15 45. The method of claim 41, wherein said cell is an abnormal cell.

46. The method of claim 45, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

20 47. A kit for the culture of an animal epithelial cell, said kit comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains the culture medium of any one of claims 1, 21, 22, 23 or 34.

48. A kit for the culture of an animal epithelial cell, said kit comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains the culture medium of claim 35.

5 49. A kit for the culture of an animal epithelial cell, said kit comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains the culture medium of claim 20 and a second carrier means contains at least one component selected from the group consisting of heparin, epidermal growth factor (EGF), a fibroblast growth factor (FGF), an agent causing an increase in intracellular levels of cyclic adenosine
10 monophosphate (cAMP), and ascorbic acid.

50. A composition comprising the culture medium of any one of claims 1, 21, 23 or 34 and an animal epithelial cell.

15 51. The composition of claim 50, wherein said animal epithelial cell is selected from the group of animal epithelial cells consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell and a tracheal epithelial cell.

52. The composition of claim 51, wherein said cell is a human cell.

53. The composition of claim 51, wherein said cell is a normal cell.

54. The composition of claim 51, wherein said cell is an abnormal cell.

20 55. The composition of claim 54, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

56. A composition comprising heparin, EGF, a fibroblast growth factor (FGF), and an agent causing an increase in intracellular levels of cyclic adenosine monophosphate (cAMP), wherein said composition replaces an organ or gland extract in an animal cell culture medium.

5 57. The composition of claim 56, further comprising ascorbic acid.

58. The composition of claim 56, wherein said FGF is selected from the group consisting of FGF-1 (aFGF), FGF-2 (bFGF) and FGF-7 (KGF).

59. The composition of claim 58, wherein said FGF is aFGF.

10 60. The composition of claim 56, wherein said agent causing an increase in intracellular levels of cAMP functions through interaction with a cellular G-protein.

61. The composition of claim 56, wherein said agent causing an increase in intracellular levels of cAMP functions by directly increasing intracellular cAMP levels.

15 62. The composition of claim 56, wherein said agent causing an increase in intracellular levels of cAMP functions by inhibiting a cAMP phosphodiesterase.

63. The composition of claim 56, wherein said agent causing an increase in intracellular levels of cAMP is a β -adrenergic receptor agonist.

20 64. The composition of claim 60, wherein said agent is cholera toxin or forskolin.

65. The composition of claim 61, wherein said agent is dibutyl cAMP.

66. The composition of claim 62, wherein said agent is isobutylmethylxanthine or theophylline.

5 67. The composition of claim 63, wherein said agent is isoproterenol.

68. The composition of claim 56, wherein said composition is a 1X-1000X concentrated formulation.

69. The composition of claim 56, wherein said composition is a 1X concentrated formulation.

10 70. The composition of claim 56, wherein said composition is a 100X concentrated formulation.

71. The composition of claim 56, wherein said composition is a 500X concentrated formulation.

15 72. The composition of claim 56, wherein said composition is a 1000X concentrated formulation.